

# Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters

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**Abstract** After the ingestion of a fat-rich meal, there is a postprandial increase in the plasma concentration of both apolipoprotein B-48- and apoB-100-containing triglyceride-rich lipoproteins (apoB-48 and apoB-100 TRL). In order to determine the contribution of these lipoproteins to postprandial lipemia, the concentration of triglycerides (TG) and retinyl esters (RE) was measured in apoB-48 and apoB-100 TRL after an oral fat load. Six normolipidemic male subjects were fed heavy cream (1 g fat per kg body weight) containing vitamin A (3000 retinol equivalents). TRL were isolated by ultracentrifugation from plasma samples obtained at regular intervals after the meal, and apoB-100 TRL were separated from apoB-48 TRL by affinity chromatography using monoclonal antibodies. Postprandial increase in plasma TG concentration was due to an increase in TG in the TRL fraction, which in turn was predominantly (82 ± 4%) due to an increase in TG in apoB-48 TRL. Contribution of apoB-100 TRL to postprandial increase in TRL TG was 3–27% in individual subjects. ApoB-100 TRL remained a significant carrier of total plasma triglyceride in the fed state, as reflected by similar apoB-100 and apoB-48 TRL TG concentrations at 2, 4, and 6 h after the fat meal. Retinyl esters were regularly detected in apoB-100 TRL. Seventy-five (± 9) percent of the increase in TRL-RE was due to RE in apoB-48 TRL and 25 ± 9% was due to RE in apoB-100. These data suggest that RE in plasma are not always associated with apoB-48-containing lipoproteins. Furthermore, we conclude that apoB-100 TRL, as well as apoB-48 TRL, make a significant contribution to postprandial triglyceridemia.—Cohn, J. S., E. J. Johnson, J. S. Millar, S. D. Cohn, R. W. Milne, Y. L. Marcel, R. M. Russell, and E. J. Schaefer. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J. Lipid Res.* 1993. 34: 2033–2040.

**Supplementary key words** postprandial lipemia • atherogenesis

It has been proposed that the interaction of triglyceride-rich lipoproteins (TRL) of intestinal and liver origin with lipoprotein lipase and cells of the artery wall constitutes

an atherogenic process (1, 2). This concept is supported by studies showing that partially delipidated very low density lipoproteins (VLDL) and chylomicrons are able to load cultured smooth muscle cells and macrophages with esterified cholesterol (3–5). The documented in vitro atherogenicity of postprandial lipoproteins has led to numerous in vivo studies documenting the qualitative and quantitative changes of plasma lipoproteins in the fed state (as reviewed in refs. 6 and 7).

We have shown in previous studies that after the ingestion of a fat-rich meal there is a postprandial increase in the plasma concentration of both apoB-48- and apoB-100-containing TRL (8). These lipoprotein species contribute to both early (0–6 h postprandially) and late (6–12 h) changes in plasma triglyceride (TG) concentration (9). The rate of TRL apoB-100 production is also increased in the fed compared to the fasted state, as measured by the in vivo rate of incorporation of intravenously administered [<sup>3</sup>H]-L-leucine into apoB-100 in the d < 1.006 g/ml fraction of plasma (10). In order to substantiate the concept that both apoB-48 and apoB-100 TRL contribute to postprandial triglyceridemia, we have assessed the contribution of these lipoproteins to postprandial increases in the plasma concentration of TRL TG.

Subjects in the present study were also fed vitamin A in order to measure the distribution of retinyl esters (RE)

Abbreviations: TRL, triglyceride-rich lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triglyceride; RE, retinyl esters.

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between apoB-48 and apoB-100 TRL. Retinyl esters in the circulation have previously been used to identify the presence in plasma of lipoproteins of intestinal origin (chylomicrons and chylomicron remnants). The rationale for this approach (11–14) is based on the concept that dietary vitamin A is esterified in the intestine and is incorporated into the core of chylomicron particles. These lipoproteins are secreted into intestinal lymph and their component TG are hydrolyzed by lipoprotein lipase in the circulation. It is believed that the RE remain associated with chylomicrons during lipolysis and are taken up by the liver within chylomicron remnants via a receptor-mediated process. Evidence suggests that the liver does not resecret these RE, and they are either stored or resecreted as unesterified retinol bound to retinol-binding protein. As plasma exchange of RE between lipoproteins is believed to be minimal and RE are not resecreted into the circulation, it is postulated that RE in plasma are appropriate markers for lipoproteins containing apoB-48 of intestinal origin. Circumstantial evidence has been presented, however, suggesting that plasma RE are not always associated with apoB-48-containing lipoproteins (15). Postprandial changes in the plasma concentration of TRL apoB-48 are not always mimicked by changes in TRL RE concentration. Furthermore, in the fasting state and 12 h after a fat-rich meal, a significant proportion of plasma RE in normolipidemic subjects is found in the low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions, apparently unassociated with apoB-48. The second purpose of the present experiments was, therefore, to directly examine the extent of RE associated with apoB-48 in the TRL fraction.

## METHODS

### Subjects

Six healthy male subjects ( $30 \pm 1$  years, mean  $\pm$  SEM) who were of average height and weight were recruited. They had normal fasting lipid levels (cholesterol:  $4.40 \pm 0.32$  mmol/l; triglycerides:  $0.86 \pm 0.09$  mmol/l; HDL cholesterol:  $1.24 \pm 0.12$  mmol/l). They were not taking vitamin A or medications known to affect plasma lipids. All studies were conducted in the Metabolic Research Unit at the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Informed consent was obtained from all volunteers under the guidelines established by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

### Fat-feeding protocol

After a 12-h overnight fast, subjects were given an oral fat load (1 g fat/kg body weight) in the form of heavy cream, together with 3000 retinol equivalents of vitamin

A (three times the recommended daily allowance). The vitamin was given as retinyl palmitate in corn oil (P1MO, a gift from Hoffman LaRoche Inc., Nutley, NJ). Blood samples (10 ml) were obtained via a small forearm indwelling catheter prior to the fat load and at 2, 4, 6, 9, and 12 h thereafter. Blood samples were collected in tubes containing ethylenediaminetetraacetate (EDTA) to give a final concentration of 0.1% EDTA, and were protected from light with the use of aluminium foil. Water, but no food, was allowed during the course of the study.

### Lipoprotein separation

Plasma was separated from red blood cells by ultracentrifugation at 1000 *g* for 15 min at 4°C. TRL fractions (containing chylomicrons, very low density lipoproteins, and intermediate density lipoproteins) were isolated from 5 ml of plasma by a single ultracentrifugal spin (39,000 rpm, 18 h, 4°C) at  $d 1.019$  g/ml in a Beckman 50.3 Ti rotor. Fraction volumes were adjusted to 3.0 ml with normal saline and were assayed for protein by the method of Lowry et al. (16), using bovine serum albumin as a standard. Turbidity was cleared with chloroform.

Freshly isolated TRL fractions were subjected to affinity chromatography, using specific apoB-100 monoclonal antibodies 4G3 and 5E11, which do not cross-react with apoB-48 (17, 18). These antibodies have previously been used to characterize the very low density lipoprotein fraction of Type III and Type IV hyperlipoproteinemic subjects (19, 20). Antibodies (mixed together in a 1:1 protein ratio) were coupled to activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) as described previously (17). Routinely, 16 mg of 4G3 protein and 16 mg of 5E11 protein were coupled to 8 ml of activated Sepharose. Binding capacity of the gel was 0.75–1.0 mg TRL protein/ml gel.

Separation of apoB-48 and apoB-100 fractions was carried out in 1.5-ml Eppendorf tubes. Sepharose gel (1.2 ml) in phosphate-buffered saline (PBS, pH 7.4, 0.02%  $\text{NaN}_3$ ) was aliquoted into tubes and centrifuged for 5 min at 1,000 rpm. The supernate was aspirated and TRL (300  $\mu\text{g}$  protein) was added to 0.6 ml of packed gel. Tubes were vortexed gently, rocked for 1 h at room temperature on an oscillating platform, and centrifuged, and the supernate was collected (approximately 0.5 ml was retrieved). The gel was washed with 1.0 ml PBS, rocked for 15–20 min, and after centrifugation, the supernate was aspirated and added to the first supernate. The washing procedure was repeated with a second volume of PBS (final volume of fraction: 2.5 ml). This sample (the unbound fraction) contained the apoB-48 TRL. Lipoproteins bound to the gel were dissociated by the addition of 3 M Na thiocyanate (1.0 ml). Samples were rocked for 1 h at room temperature, centrifuged, and the supernate was collected. The gel was washed with two further 1.0-ml aliquots of Na thiocyanate, and these washes were added to the first

thiocyanate supernate to give the bound fraction, containing apoB-100 TRL. Recovery of initial TRL added to the affinity gel was  $92 \pm 4\%$  as estimated by measuring the recovered protein in the bound and unbound fractions.

The adequacy of lipoprotein separation was assessed by SDS polyacrylamide gel electrophoresis using 4–22.5% acrylamide gradient gels as previously described (8). Nondelipidated lipoprotein samples (30  $\mu\text{g}$  protein), reduced in SDS sample buffer containing 3% mercaptoethanol, were routinely loaded onto the 1.5-mm thick vertical slab gels. The apoB-48 (unbound) fractions were concentrated before electrophoresis to allow for adequate visualization of the apoB-48 protein band. The apoB-100 band of TRL comigrated with the major stainable protein of a narrow-cut LDL fraction ( $1.040 < d < 1.050$  g/ml). The apoB-48 band of TRL comigrated with the major high molecular weight band of lymph chylomicrons. The identity of the apoB bands was confirmed by Western blotting analysis using a polyclonal antibody that was known to react with both apoB-48 and apoB-100.

### Lipid and lipoprotein analyses

Plasma and lipoprotein fractions were assayed for total cholesterol and TG with an Abbott Diagnostics ABA-200 bichromatic analyzer using enzymatic reagents (21). Assays were standardized through participation in the Centers for Disease Control—National Heart, Lung and Blood Institute Standardization program. All lipid measurements (including separated TRL fractions) were carried out in duplicate. HDL cholesterol was quantitated by analyzing the supernate obtained by the precipitation of a plasma aliquot with dextran- $\text{Mg}^{2+}$  (22). Retinyl esters were measured under red lights by high-performance liquid chromatography using retinyl acetate as an internal standard (23). Using this method, all the major plasma RE (retinyl palmitate, stearate, and oleate) coeluted together and appeared as a single peak on the chromatograms.

### Statistics

Paired *t*-tests were used to determine the statistical significance between lipid measurements obtained from fasting (0 h) and postprandial samples. Postprandial increase in TRL TG and RE was measured by planimetry as the area under the concentration-time curve, where the baseline was the zero-time (fasting) concentration. Area measurements are expressed in the units of  $\text{mmol} \cdot \text{h/l}$ .

## RESULTS

Mean ( $\pm$  SEM) plasma cholesterol, TG, and RE concentrations before and after the ingestion of the fat load are shown in Fig. 1. Mean plasma cholesterol concentration did not change significantly during the course of the experiment. This is consistent with our previous studies

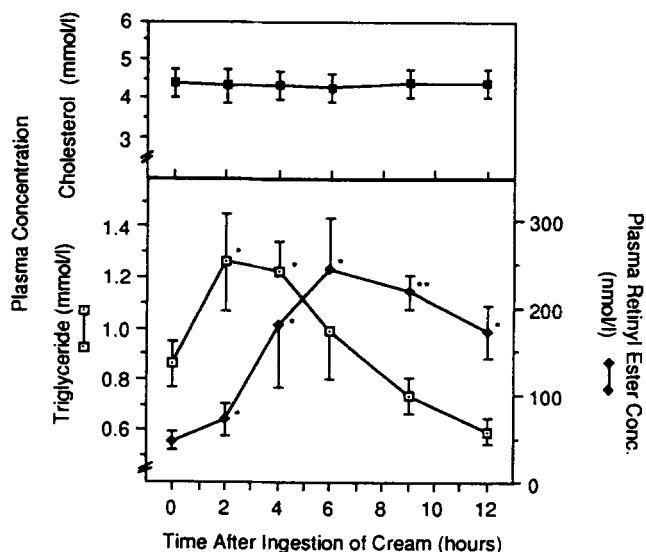
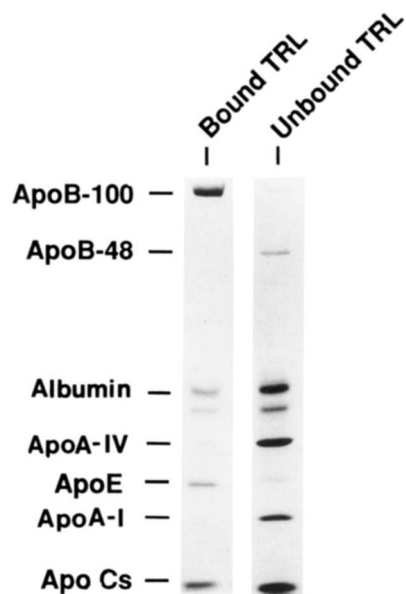


Fig. 1. Postprandial changes in the plasma concentration of cholesterol, triglycerides, and retinyl esters. Significantly different from fasting concentration by paired *t*-test: \* $P < 0.05$ ; \*\* $P < 0.01$ .

(24) and those of others (6). Plasma TG concentration peaked either 2 or 4 h after the fat load in individual subjects, and the mean plasma TG concentration was significantly ( $P < 0.05$ ) elevated above fasting concentration 2 and 4 h postprandially. Mean plasma RE concentration peaked at 6 h and was significantly greater than baseline at all postprandial timepoints. As shown previously (9, 15) and as reflected by the different time course of the curves for the mean data (Fig. 1), change in plasma RE concentration occurred after change in plasma TG in all subjects.

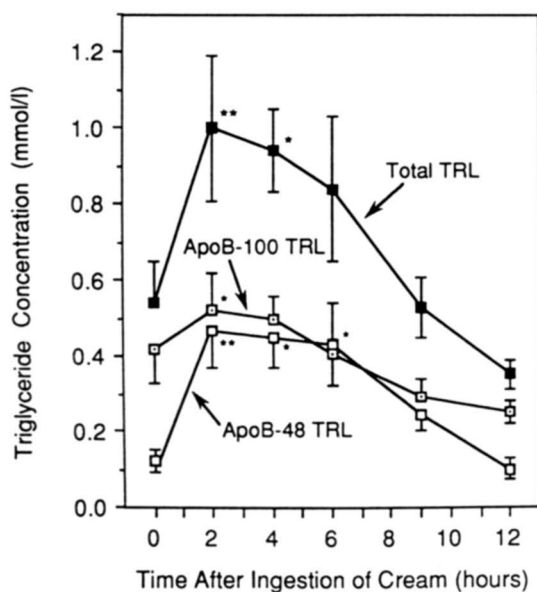
TRL fractions ( $d < 1.019$  g/ml) were isolated by ultracentrifugation and were separated into apoB-48 and apoB-100 TRL species by affinity chromatography. The apolipoprotein composition of bound and unbound fractions is shown in Fig. 2. Very adequate separation of apoB-48- and apoB-100-containing fractions was achieved. ApoB-48 was not detectable in the bound (apoB-100-containing) fractions. A faint apoB-100 band was often observed in the unbound fractions (see right hand gel of Fig. 2). Minor contamination of the unbound fraction has been noted previously using these antibodies (19, 20). Additional chromatography was not carried out to remove contaminating apoB-100 material, as this was found to adversely affect recovery. Laser scanning densitometry showed that less than 1% of the apolipoprotein composition of apoB-48 TRL was apoB-100 (assuming equal chromogenicity of apolipoprotein bands). Two percent of apoB-48 TRL protein was apoB-48, 15% was albumin, 8% was  $\beta$ -2-glycoprotein 1, 21% was apoA-IV, 2% was apoE, 19% was apoA-I, and 34% was C-apolipoproteins. In apoB-100 TRL, 35% of total protein by gel scanning was apoB-100, 15% was albumin, 10%



**Fig. 2.** Apolipoprotein composition of TRL fractions separated by affinity chromatography using monoclonal antibodies against apoB-100. The bound fraction containing apoB-100 is on the left, and the unbound fraction containing apoB-48 is on the right. Bound TRL contained relatively more apoE and less apoA-IV and apoA-I than unbound TRL. The unbound fraction was concentrated before loading onto the gel. Trace amounts of apoB-100 were routinely detected in the unbound fraction.

was  $\beta$ -2-glycoprotein 1, 1% was apoA-IV, 9% was apoE, and 31% was C-apolipoproteins.

In the fasting state (at zero time), mean plasma TG concentration was  $0.86 \pm 0.09$  mmol/l and mean TRL TG concentration was  $0.54 \pm 0.11$  mmol/l (representing  $61 \pm 6\%$  of total plasma triglyceride). In the fed state at



**Fig. 3.** Postprandial changes in the triglyceride concentration of TRL fractions. Significantly different from fasting concentration by paired *t*-test: \**P* < 0.05; \*\**P* < 0.01.

the timepoint of maximal TG increase (2 h postprandially), mean plasma TG concentration was  $1.26 \pm 0.19$  mmol/l and mean TRL TG concentration was  $1.00 \pm 0.19$  mmol/l. A mean  $77 \pm 3\%$  of plasma TG was thus contained in the TRL fraction 2 h after the fat load. Mean TRL TG was significantly increased above fasting concentration 2 and 4 h after the fat load (**Fig. 3**) in accord with the total plasma TG data. These results are comparable to those obtained in male subjects of different ages, reported previously (8).

The plasma TG concentration of the apoB-48 and apoB-100 fractions before and after the fat load is shown in Fig. 3. Postprandial increase in plasma TG concentration was predominantly due to increase in apoB-48 TRL, which was significantly increased 2, 4, and 6 h after the meal. A smaller but significant increase was observed in apoB-100 TRL TG. The contribution of apoB-48 and apoB-100 TRL fractions to postprandial increase in TRL TG (**Table 1**) was quantitated by measuring the area under individual TG response curves (fasting [0 h] concentration taken as baseline). Increase in apoB-48 TRL TG was five times greater than increase in apoB-100 TRL TG ( $2.44 \pm 0.45$  vs.  $0.47 \pm 0.15$  mmol  $\cdot$  h/l). Eighty-two ( $\pm 4$ ) percent of the postprandial increase in TRL TG was thus due to apoB-48 TRL (range: 73–97%). The contribution of apoB-100 TRL was not insignificant, accounting for 3–27% of total increase in TRL TG in individual subjects (mean  $\pm$  SEM:  $18 \pm 4\%$ ). Total area (as opposed to incremental area) was also measured under the TRL TG response curves (zero concentration used as baseline). The apoB-48 TRL TG total area was  $3.96 \pm 0.48$  mmol  $\cdot$  h/l and the apoB-100 TRL TG area was  $4.64 \pm 0.75$  mmol  $\cdot$  h/l. This means that although apoB-48 TRL were most responsible for the increase in TRL TG concentration after the fat load, in absolute terms apoB-100 TRL were the predominant carriers of plasma TG during the total 12 h postprandial period.

RE were measured in TRL fractions isolated at different postprandial timepoints. It is significant that we detected RE in nearly all apoB-100 TRL, as well as apoB-48 TRL samples. The mean plasma RE concentrations of

**TABLE 1.** Contribution of apoB-100 TRL and apoB-48 TRL to postprandial increases in the plasma concentration of triglyceride and retinyl ester

Increase in Concentration <sup>a</sup>	ApoB-100 TRL	ApoB-48 TRL
mmol $\cdot$ h/l		
Triglyceride	$0.47 \pm 0.15$ (18%) <sup>b</sup>	$2.44 \pm 0.45$ (82%) <sup>b</sup>
Retinyl ester	$333 \pm 129$ (25%) <sup>b</sup>	$889 \pm 169$ (75%) <sup>b</sup>

<sup>a</sup> Measured as the area between the postprandial response curves and a baseline drawn through the fasting concentration.

<sup>b</sup> Values in parentheses represent increases expressed as a percentage of the total.

the total, apoB-48, and apoB-100 TRL fractions before and after the fat load are shown in Fig. 4. Total TRL RE concentration was significantly elevated 2, 4, 6, and 9 h after the fat load, which mimicked the postprandial changes in total plasma RE concentration (Fig. 1). Increases in both apoB-48 TRL and apoB-100 TRL RE were responsible for the change in total TRL RE. RE concentration peaked on average at 6 h in the apoB-48 TRL fraction, whereas the maximum RE concentration in apoB-100 TRL tended to occur at later timepoints (at 9 h for the mean data). The contribution of apoB-100 TRL and apoB-48 TRL to postprandial increases in plasma TRL RE concentration was quantitated by planimetry and mean area measurements are shown in Table 1. Increase in RE concentration postprandially tended to be 2- to 3-times greater in apoB-48 TRL than in apoB-100 TRL. Thus  $75 \pm 9\%$  of TRL RE increase was due to apoB-48 TRL and  $25 \pm 9\%$  was due to apoB-100 TRL. It is worth noting that the contribution of apoB-100 TRL varied from one subject to another, and was quite significant in some individuals (range: 6–56%).

Cholesterol concentrations were also measured in the different TRL fractions and these results are shown in Fig. 5. A postprandial increase in total TRL cholesterol was not observed in every subject (as we have observed in previous studies (8)). In this group of subjects, who had relatively low plasma cholesterol concentrations, a decrease in the mean total TRL cholesterol below fasting concentration was, in fact, observed at later timepoints. This decrease was predominantly due to a fall in the postprandial concentration of apoB-100 TRL cholesterol. In

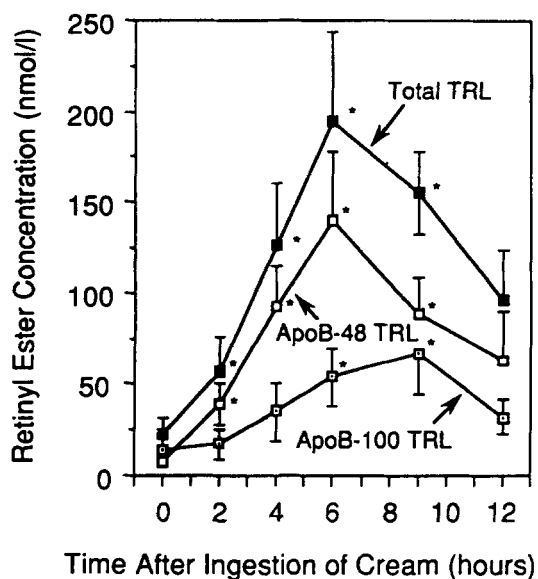


Fig. 4. Postprandial changes in the retinyl ester concentration of TRL fractions. Significantly different from fasting concentration by paired *t*-test: \**P* < 0.05.

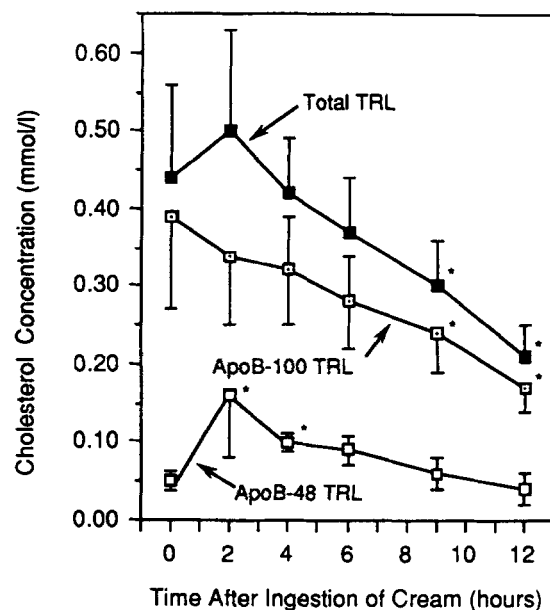


Fig. 5. Postprandial changes in the cholesterol concentration of TRL fractions. Significantly different from fasting concentration by paired *t*-test: \**P* < 0.05.

contrast, mean apoB-48 TRL cholesterol was significantly increased 2 and 4 h after the fat load.

## DISCUSSION

Specific monoclonal antibodies for apoB-100 have been used before (19, 20) to isolate and characterize the very low density lipoprotein (VLDL) fraction of dyslipidemic subjects in the fasted state. The same antibodies were used in the present study to isolate the apoB-48- and apoB-100-containing TRL of plasma obtained from subjects in the fed state. In order to obtain reliable quantitative data with these antibodies, we have separated single sample aliquots in individual Eppendorf tubes (see Methods), containing antibodies bound to Sepharose, rather than eluting fractions from affinity chromatography columns, as described previously (19). In addition, sodium thiocyanate rather than citric acid was used to separate the bound TRL fraction from the affinity chromatography gels. These modifications resulted in acceptable recovery (85–95%) of TRL samples and allowed for the plasma concentration of different fractions to be estimated. Adequacy of separation was also controlled as closely as possible by monitoring the apolipoprotein composition of isolated fractions by polyacrylamide gel electrophoresis (Fig. 2). Some contamination of apoB-48 fractions was noted (see Methods), leading to a small underestimation of apoB-100 TRL lipid concentrations. This underestimation, if taken into account, would only

increase the contribution of apoB-100 TRL and enhance the significance of the present results.

We have shown that increase in plasma triglyceride concentration after an oral fat load is predominantly due to triglyceride contained within apoB-48-containing lipoproteins in the TRL fraction of plasma. In normolipidemic male subjects, we have found that  $82 \pm 4\%$  of the postprandial increase in TRL TG was attributable to triglyceride in apoB-48 TRL. This is consistent with the established concept that dietary fat is incorporated into large triglyceride-rich chylomicrons in the intestine that have apoB-48 as their major structural apolipoprotein (25). These lipoproteins are secreted into intestinal lymph and then into the circulation where their triglycerides are hydrolyzed by lipoprotein lipase. ApoB-48-containing lipoproteins in the circulation, therefore, represent a spectrum of partially catabolized lipoproteins of intestinal origin, which make a significant contribution to postprandial triglyceridemia.

Although the increase in plasma TRL TG concentration in the fed state was predominantly due to an increase in apoB-48 TRL, the contribution of apoB-100 TRL was not insignificant;  $18 \pm 4\%$  of the total TRL TG increase following the oral fat load was attributable to apoB-100 TRL, and this varied among individual subjects (range: 3–27%). In absolute terms, apoB-100 TRL were responsible for transporting a significant proportion of plasma TG in the postprandial state, as reflected by the relatively similar concentrations of TG in apoB-100 and apoB-48 TRL at 2, 4, and 6 h after the fat load (Fig. 3), and as reflected by the total area under the TRL response curves for the 12-h period after the meal ( $4.64 \pm 0.75$  vs.  $3.96 \pm 0.48$  mmol · h/l for apoB-100 TRL and apoB-48 TRL, respectively). Similar data have recently been presented by Schneeman et al. (26). These results suggest that apoB-100 TRL make a significant contribution to the total plasma triglyceride concentration in the fed as well as in the fasted state.

There are at least three possible explanations for the increase in the concentration of apoB-100 TRL triglyceride in postprandial plasma. 1) The plasma clearance of apoB-100 TRL is inhibited by the influx into plasma of postprandial chylomicrons; 2) the ingestion of a fat-rich meal stimulates the synthesis and secretion of apoB-100 TRL from the intestine; and 3) hepatic apoB-100 TRL are secreted postprandially in response to lipid of dietary origin reaching the liver via the portal vein or via chylomicron transport. The first possibility is supported by the studies of Brunzell et al. (27) showing that chylomicrons and VLDL are catabolized by a common pathway. Triglycerides in both chylomicrons and VLDL are hydrolyzed by lipoprotein lipase at the capillary endothelial surface and these lipoproteins can compete for enzyme-mediated lipolysis. Reduced availability of lipolytic activity due to the postprandial presence of apoB-48-

containing chylomicrons could explain the observed increase in apoB-100 TRL triglyceride. An increase in the production of apoB-100 TRL is, however, an equally possible explanation. We have previously found that in the fed state there is an increase (50% on average) in the plasma concentration of apoB-100 in the TRL fraction (8), as well as an increase in the rate of production of TRL apoB-100 (10). We have suggested that this represents a postprandial increase in postprandial synthesis and secretion of TRL by the liver, as it is normally assumed that apoB-100 in plasma is of hepatic origin. Data from a recent study in which apoB-100 epitopes were determined in human subjects before and after liver transplantation (28) support the hepatic origin of circulating apoB-100. Other lines of evidence, however, have suggested that the human intestine has the capacity to synthesize apoB-100 (29, 30), and it cannot be ruled out that in the fed state apoB-100 in TRL is partly of intestinal origin.

Irrespective of the source of apoB-100 TRL in the fed state, and irrespective of the mechanism for the increased presence of apoB-100 TRL in postprandial plasma, our studies suggest an additional reason why the postprandial state is potentially atherogenic. Zilversmit (1) originally proposed that atherogenesis was a “postprandial phenomenon” because it involved the “binding of chylomicrons to the arterial surface, the hydrolysis of triglyceride by arterial lipoprotein lipase and the subsequent internalization of cholesterol-enriched chylomicron remnants by arterial smooth muscle cells.” Postprandial apoB-100 TRL may, however, be of equal significance, especially since a certain proportion of TRL containing apoB-100 are the precursors of potentially atherogenic LDL (31) and partially degraded apoB-100 TRL remnants have themselves been shown to be atherogenic (2).

An important finding of the present study is that RE were routinely detected in apoB-100 TRL. The presence of RE in apoB-100 TRL was of greater significance at later postprandial timepoints (Fig. 4), and for the 12-h period as a whole  $25 \pm 9\%$  of TRL RE was contributed by RE in apoB-100 TRL. These data lend support to the circumstantial evidence that we and others have presented previously, suggesting that plasma RE are not always associated in plasma with apoB-48-containing lipoproteins (12, 15, 32). There are three possible explanations for the presence of RE in apoB-100 TRL. 1) RE are secreted by the intestine within chylomicrons containing apoB-100 or possibly within smaller “VLDL-sized” apoB-100 particles; 2) RE are secreted by the liver in VLDL containing apoB-100; or 3) RE are transferred to apoB-100 TRL in the circulation from other plasma lipoproteins. As discussed before, evidence has been presented showing that the intestine has the capacity to synthesize apoB-100 (29, 30). The quantitative significance of this synthesis and whether it results in the secretion of a mature protein remains, however, to be substantiated. As far as hepatic

secretion of RE is concerned, there are data from isolated cell studies (33) and from experimental animals (34) that suggest that RE are not secreted by the liver. Nevertheless, it cannot be totally ruled out that the human liver in vivo is able to secrete RE incorporated into apoB-100-containing lipoproteins. The third possibility is supported by evidence that small amounts of RE are able to transfer between plasma lipoproteins (35, 36); however several investigators consider this transfer to be quantitatively insignificant (12, 14, 37).

Finally, plasma RE have been used as a measure of the duration and extent of postprandial lipemia in order to establish the role of postprandial lipoproteins (specifically intestinal lipoproteins) in the etiology of atherosclerosis (38-40). As suggested before, however (15), studies tracing the presence of RE in plasma for relatively long periods of time after meal-feeding need to be interpreted with caution. Small amounts of RE not associated with the TRL fraction (associated with LDL and HDL at later timepoints) are probably not associated with remnant lipoproteins. In addition, RE within the TRL fraction are not always associated with apoB-48-containing lipoproteins and they are therefore not necessarily indicative of TRL of intestinal origin. ■

Supported by grant HL 39326 from the National Institutes of Health and contract 53-3K-06 from the U.S. Department of Agriculture Research Service.

Manuscript received 19 July 1991, in revised form 26 May 1993, and in re-revised form 21 July 1993.

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